09/582877

534 Rec'd PCT/PTO 30 JUN 2000

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

REQUEST FOR FILING NATIONAL PHASE OF PCT APPLICATION UNDER 35 U.S.C. 371 AND 37 CFR 1.494 OR 1.495

To: Asst. Commissioner of Patents and Trademarks
Washington, D.C. 20231

(Our Deposit Account No. 03-3975

TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US)

Atty Dkt: PM 271427 /00-PSBUS-574
M# /Client Ref.

From: Pillsbury Madison & Sutro LLP, IP Group:

Date: June 30, 2000

This is a **REQUEST** for **FILING** a PCT/USA National Phase Application based on:

1. International Application	2. International Filing Date	3. Earliest Priority Date Claimed
PCT/KR99/00659	3 November 1999	3 November 1998
⇧ country code	Day MONTH Year	Day MONTH Year
		(use item 2 if no earlier priority)

4. Measured from the earliest priority date in item 3, this PCT/USA National Phase Application Request is being filed within:

(a) ☒ 20 months from above item 3 date (b) ☐ 30 months from above item 3 date,

(c) Therefore, the due date (unextendable) is July 3, 2000

5. Title of Invention PHARMACEUTICAL COMPOSITION HAVING ANTITUMOR ACTIVITY AND PROCESS FOR THE PREPARATION THEREOF

6. Inventor(s) KIM, Song-Bae

Applicant herewith submits the following under 35 U.S.C. 371 to effect filing:

7. ☒ Please immediately start national examination procedures (35 U.S.C. 371 (f)).

8. ☐ **A copy of the International Application** as filed (35 U.S.C. 371(c)(2)) is transmitted herewith (file if in English but, if in foreign language, file only if not transmitted to PTO by the International Bureau) including:

a. ☐ Request;
b. ☐ Abstract;
c. _____ pgs. Spec. and Claims;
d. _____ sheet(s) Drawing which are ☐ informal ☐ formal of size ☐ A4 ☐ 11"

9. ☒ **A copy of the International Application has been transmitted by the International Bureau.**

10. **A translation of the International Application** into English (35 U.S.C. 371(c)(2))

a. ☒ is transmitted herewith including: (1) ☒ Request; (2) ☒ Abstract;
(3) 22 pgs. Spec. and Claims;
(4) _____ sheet(s) Drawing which are:
☐ informal ☐ formal of size ☐ A4 ☐ 11"

b. ☐ is not required, as the application was filed in English.

c. ☐ is not herewith, but will be filed when required by the forthcoming PTO Missing Requirements Notice per Rule 494(c) if box 4(a) is X'd or Rule 495(c) if box 4(b) is X'd.

d. ☐ Translation verification attached (not required now).

RE: USA National Filing of PCT/KR99/00659

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11. ☒ **PLEASE AMEND** the specification before its first line by inserting as a separate paragraph:
a. ☒ --This application is the national phase of international application PCT/KR99/00659 filed November 3, 1999 which designated the U.S.--
b. ☐ --This application also claims the benefit of U.S. Provisional Application No. 60/_____, filed _____.--

12. ☐ Amendments to the claims of the International Application **under PCT Article 19 (35 U.S.C. 371(c)(3)), i.e., before 18th month from first priority date above in item 3, are transmitted herewith (file only if in English) including:**

13. ☒ PCT Article 19 claim amendments (if any) have been transmitted by the International Bureau

14. ☐ Translation of the amendments to the claims **under PCT Article 19 (35 U.S.C. 371(c)(3)), i.e., of claim amendments** made before 18th month, **is attached (required by 20th month from the date in item 3 if box 4(a) above is X'd, or 30th month if box 4(b) is X'd, or else amendments will be considered canceled).**

15. **A declaration of the inventor** (35 U.S.C. 371(c)(4))
a. ☐ is submitted herewith ☐ Original ☐ Facsimile/Copy
b. ☒ is not herewith, but will be filed when required by the forthcoming PTO Missing Requirements Notice per Rule 494(c) if box 4(a) is X'd or Rule 495(c) if box 4(b) is X'd.

16. **An International Search Report (ISR):**
a. Was prepared by ☐ European Patent Office ☐ Japanese Patent Office ☒ Other
b. ☒ has been transmitted by the international Bureau to PTO.
c. ☒ copy herewith (1 pg(s).) ☒ plus Annex of family members (1 pg(s).).

17. **International Preliminary Examination Report** (IPER):
a. ☒ has been transmitted (if this letter is filed after 28 months from date in item 3) in English by the International Bureau with Annexes (if any) in original language.
b. ☐ copy herewith in English.
c.1 ☐ IPER Annex(es) in original language ("Annexes" are amendments made to claims/spec/drawings during Examination) including attached amended:
c.2 ☐ Specification/claim pages #_____ claims #
Dwg Sheets #
d. ☐ Translation of Annex(es) to IPER **(required by 30th month due date, or else annexed amendments will be considered canceled).**

18. **Information Disclosure Statement** including:
a. ☒ Attached Form PTO-1449 listing documents
b. ☐ Attached copies of documents listed on Form PTO-1449
c. ☒ A concise explanation of relevance of ISR references is given in the ISR.

19. ☐ **Assignment** document and Cover Sheet for recording are attached. Please mail the recorded assignment document back to the person whose signature, name and address appear at the end of this letter.

20. ☐ Copy of Power to IA agent.

21. ☐ **Drawings** (complete only if 8d or 10a(4) not completed): ___ sheet(s) per set: ☐ 1 set informal; ☐ Formal of size ☐ A4 ☐ 11"

22. ☐ _____ (No.) **Verified Statement(s)** establishing "small entity" status under Rules 9 & 27

23. **Priority** is hereby claimed under 35 U.S.C. 119/365 based on the priority claim and the certified copy, both filed in the International Application during the international stage based on the filing in (country) SOUTH KOREA of:

	Application No.	Filing Date		Application No.	Filing Date
(1)	1998-47025	Nov. 3, 1998	(2)	1998-48277	Nov. 11, 1998
(3)			(4)		
(5)			(6)		

a. ☒ See Form PCT/IB/304 sent to US/DO with copy of priority documents. If copy has not been received, please proceed promptly to obtain same from the IB.
b. ☐ Copy of Form PCT/IB/304 attached.

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24. Attached:

25. **Preliminary Amendment:**

25.5 Per Item 17.c2, **cancel original** pages #_____, claims #_____, Drawing Sheets #

26. **Calculation of the U.S. National Fee (35 U.S.C. 371 (c)(1)) and other fees is as follows:**
Based on amended claim(s) per above item(s) ☐ 12, ☐ 14, ☐ 17, ☐ 25, ☐ 25.5 (hilite)

Total Effective Claims	minus 20 =	x $18/$9	=	$0	966/967
Independent Claims	minus 3 =	x $78/$39	=	$0	964/965
If any proper (ignore improper) Multiple Dependent claim is present,		add$260/$130		+0	968/969

BASIC NATIONAL FEE (37 CFR 1.492(a)(1)-(4)): →→ **BASIC FEE REQUIRED, NOW** →→→→

A. If country code letters in item 1 are **not** "US","BR","BB","TT","MX","IL" "NZ", "IN" or "ZA"

See item 16 re:

1.	Search Report was not prepared by EPO or JPO	add$970/$485		960/961
2.	Search Report was prepared by EPO or JPO	add$840/$420	+970	970/971

SKIP B, C, D AND E UNLESS country code letters in item 1 are "US","BR","BB","TT","MX","IL", "NZ", "IN" or "ZA"

(X) →	☐	B.	If USPTO did not issue both International Search Report (ISR) and (if box 4(b) above is X'd) the International Examination Report (IPER),	add$970/$485	+0	960/961
(only) (one) → (of)	☐	C.	If USPTO issued ISR but not IPER (or box 4(a) above is X'd),	add$690/$345	+0	958/959
(these) (4) → (boxes)	☐	D.	If USPTO issued IPER but IPER Sec. V boxes not all 3 YES,	add$670/$335	+0	956/957
→	☐	E.	If international preliminary examination fee was paid to USPTO and Rules 492(a)(4) and 496(b) satisfied (IPER Sec. V all 3 boxes YES for all claims),	add $96/$48	+0	962/963

27.		**SUBTOTAL =**	$970
28.	If Assignment box 19 above is X'd, add Assignment Recording fee of ----$40	+0	(581)
29.	Attached is a check to cover the	**TOTAL FEES**	$970

Our Deposit Account No. 03-3975
Our Order No. 71404 (C#) | 271427 (M#)

CHARGE STATEMENT: The Commissioner is hereby authorized to charge any fee specifically authorized hereafter, or any missing or insufficient fee(s) filed, or asserted to be filed, or which should have been filed herewith or concerning any paper filed hereafter, and which may be required under Rules 16-18 and 492 (missing or insufficient fee only) now or hereafter relative to this application and the resulting Official document under Rule 20, or credit any overpayment, to our Account/Order Nos. shown above for which purpose a duplicate copy of this sheet is attached.
This CHARGE STATEMENT does not authorize charge of the issue fee until/unless an issue fee transmittal form is filed

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NOTE: File in duplicate with 2 postcard receipts (PAT-103) & attachments.

Pharmaceutical composition having antitumor activity and process for the preparation thereof

Field of the invention

The present invention relates to a pharmaceutical composition having antitumor activity which contains herb medicines as the main ingredients, and process for the preparation thereof.

Prior arts

The inventor had invented a pharmaceutical composition of herb medicines having antitumor activity and process for the preparation thereof, and the invention was granted the Korean patent No. 72982.

The above-mentioned patent discloses a pharmaceutical composition containing Pulsatillae Radix (Pulsatilla koreana Nakai, P. cernua, P. danurica, P. ratensis, Chinese Pulsatillae, Mongolian Pulsatillae) and/or Clematis Chinensis Osbeclo (so called, Chinese clematis) as the main ingredients, and optionally Ulmaceae Cortex, Armeniacae Semen, Ginseng Radix and Glycyrrhizae Radix and process for the preparation thereof.

Pulsatillae species are grown wild all over the world, and the Pulsatillae Radix has been used as an antiphlogistic agent, astringent and hemostatic agent and thus for the treatment of dysentery in Korea. It is known that the Pulsatillae Radix contains anemonin, protoanemonin and saponin. Protoanemonin is the precursor of anemonin, and both may be dissolved in water, alcohol, chloroform, methylene chloride and the like.

Clematis Radix contains anemonin, anemonol and saponin. It has been used as an agent for gout, diuretic agent and agent for difficult menstruum.

Ulmaceae Cortex contains mucin and tannin, and has been used as a lenitive and adhesive.

Ginseng Radix has been known from ancient times as a marvellous medicine in the Far East. It has been used as a tonic, agent for acute gastritis and agent for various bleeding diseases. Recently, it is reported that Ginseng Radix has antitumor activity and contains Ginseng alkaloids, Ginseng saponins, essence oil, etc.

Glycyrrhizae Radix contains glycyrrhizin, liquiritin, licoricidin and liquiritoside and has been used as a cough remedy, expectorant, diaphoretic and agent for gastritis.

The above invention by the present inventor relates to a pharmaceutical composition having excellent antitumor activity and containing extract or powder of Pulsatillae Radix and/or Clematis Radix as the main ingredients, and optionally extract or powder of Ulmaceae Cortex, Armeniacae Semen, Ginseng Radix and Glycyrrhizae Radix.

By the method of the prior invention, the composition may be prepared by drying and finely powdering each herb ingredients ; by extracting the herb ingredients in a solvent selected from water, lower alcohol, chloroform, methylene chloride and the others which may dissolve the effective ingredients of the herbs at the temperature of 0℃ - the boiling point of the used solvent for 30 minutes to 24 hours and then vaporizing the used solvent to give the extract ; or dissolving said extract in water, alcohol or the mixed solvent thereof. When extracting the effective ingredients, each herb may be extracted independently or

two or more herbs may be extracted together. Then, the extract is powdered and formulated to a pharmaceutical preparation by using vehicles such as lactose, various starches, sucrose, mannitol, sorbitol and inorganic salts such as calcium phosphate, calcium sulfate, aluminiun silicate and calcium carbonate ; binders such as sucrose, glucose, starch, gelatin, carboxymethylcelluose, methylcellulose, gum arabic, gum tragacanth, ethylcellulose, sodium alginate, hydroxypropylmethylcellulose, polyvinylpyrrolidone and soluble cellulose ; disintegrators such as starch, carboxymethylcellulose, methylcellulose and crystalline cellulose ; lubricants such as magnesium stearate and calcium stearate ; wetting agents such as glycerine, propylene glycol and sorbitol ; preservatives such as sodium benzoate, methyl p-hydroxybenzoate, propyl p-hydroxybenzoate, benzalkonium chloride, chlorobutanol and sodium dehydroacetate ; dissolving agents such as soluble alcohols and derivatives thereof, and various surfactants ; antioxidants such as sodium sulfite, sodium pyrosulfate, sodium metasulfate, sodium bisulfite, rongalite and ascorbic acid ; isotonic agents such as sodium chloride and dextrose ; indolent agents such as benzylalcohol and chlorobutanol ; and ointment bases such as vaseline, fluid paraffin, various vegetable oils, waxes and lanoline ; and other conventional auxiliary vehicles or carriers.

The inventor has continued intensive study to improve the antitumor composition and found that the composition by the prior invention is very unstable for preservation whereby it may easily lose its pharmaceutical effects in 3-6 months.

Summary of the invention

Accordingly, an object of the present invention is to provide an improved pharmaceutical composition which is stable and maintains its pharmaceutical efficacy even if it is preserved for several years, comprising lyophilized powder of Pulsatillae Radix, Ulmaceae Cortex, or mixture thereof as the main herb ingredients, and optionally one or more auxiliary herb ingredients selected from Ginseng Radix and Glycyrrhizae Radix, and conventional auxiliaries such as used in the prior invention (Korean patent No. 72982).

Particularly, the inventor has completed the present invention by discovering that herb ingredients should be extracted in a solvent at the temperature of below 60℃ and promptly lyophilized in order to maintain efficacy of the composition and preserve it for a long time.

Detailed description of the invention

The pharmaceutical composition in the present invention comprises as the main active herb ingredients 0-100wt% of Pulsatillae Radix and 0-100wt% of Ulmaceae Cortex, and optionally as the auxiliary herb ingredients 0-70wt% of Ginseng Radix and 0-70wt% of Glycyrrhizae Radix, wherein the contents are in terms of dried herb ingredients. Preferably the content of Pulsatillae Radix and/or Ulmaceae Cortex is over 30wt%.

The pharmaceutical composition having antitumor activity according to the present invention may be prepared by :

extracting powdered Pulsatillae Radix and/or powdered Ulmaceae cortex,

and optionally one ore more herb ingredients selected from powdered Ginseng Radix and powdered Glycyrrhizae Radix in a solvent at the temperature of below 60℃, filtering and lyophilizing the extract, and admixing the lyophilized powder with conventional auxiliaries, alternatively admixing the above extracted solution with auxiliaries, then filtering and lyophilizing the mixture, and then formulating the lyophilized powder to a pharmaceutical preparation by a conventional method used in the pharmaceutics.

In case the composition is to be used by injection, before lyophilization, it is advisable that the extracted solution is premixed with conventional auxiliaries including a preservative such as methylparaben, ethylparaben and propylparaben, an isotonic agent such as sodium chloride and an indolent agent such as benzylalcohol.

In case the composition is to be used in a form of capsule, tablet, ointment or the like except the injection, the extracted solution of the herb ingredients is lyophilized and then the lyophilized powder is admixed with conventional auxiliaries such as used in the invention of the Korean patent No. 72982 by a conventional method in the pharmaceutics to give a pharmaceutical preparation.

The solvent for extraction of herb ingredients may include water, lower alcohol, acetone, ethyl acetate, hexane and mixtures thereof.

The herb ingredients are extracted in a solvent at the temperature of below 60℃, and immediately lyophilized. The lyophilized powder of the herb ingredients as above may be filled into a vial and it may be

applied by adding distilled water for injection thereinto, or the lyophilized powder may be formulated to a form of capsule, tablet or ointment by a conventional method in the pharmaceutics.

About 100mg to 5g of the present composition on the basis of the lyophilized powder may be administered in a day, once a week to 1 - 3 times for a day. The dose of the composition may be varied in consideration of sex, age, condition of disease, etc. of the patients

The present invention will be explained in more detail with the following examples and experiments.

Comparative example 1

6.26g of powdered Pulsatillae Radix was added into 90ml of purified water and the mixture was warmed to 60℃ and stirred for 60 minutes, and then centrifuged at 3,500 rpm for 30 minutes. 50ml of the centrifuged solution was filtered in a sterilized room at below 60℃. The resulting solution was made to isotonic solution by adding NaCl under the aseptic condition, then sterile-filtered once again and divided to each 2.5 ml of the solution in an ampoule of 3ml under the aseptic condition, and sealed to obtain injection ampoules.

Comparative example 2

4g of powdered Pulsatillae Radix, 2g of powdered Ulmaceae cortex, 2g of powdered Ginseng Radix and 1g of powdered Glycyrrhizae Radix were added to 90ml of purified water and the mixture was stirred for 60 minutes at about 80℃ by adding purified water corresponding to the water distilled off. The resulting solution was cooled to room

temperature, centrifuged with 3,500 rpm for 30 minutes to obtain 46ml of the extracted solution. NaCl was added to the extract to obtain isotonic solution. The isotonic solution was filtered by a conventional method in a sterilized room, sterile-filtered and divided into each 2ml of the solution in an ampoule of 3ml, sealed and stored in a refrigerator.

Comparative example 3

62.6g of powdered Pulsatillae Radix, 31.3g of powdered Ginseng Radix and 10g of powdered Glycyrrhizae Radix were added to 900ml of purified water and extracted for 60 minutes at about 60℃ with adding purified water corresponding to the water distilled off. The resulting solution of 40ml was filtered and concentrated to give 26.4g of the concentrated extract.

Example 1

6g of powdered Pulsatillae Radix was added into 100ml of distilled water and extracted for 60 minutes at below 60℃ with stirring. After the extract was centrifuged with 5000 rpm, 900mg of NaCl as an isotonic agent and 160mg of methyl paraoxybenzoate were added thereto, and sterile-filtered in a sterilized room, divided into 20 vials of 5ml, promptly lyophilized at below -40℃ and sealed to obtain injectable powder.

Example 2

6g of powdered Pulsatillae Radix, 4g of powdered Ulmaceae cortex and 0.9g of powdered Glycyrrhizae Radix were added to 100ml of distilled water and extracted for 60 minutes at a temperature of below 60℃ with stirring. After the extracted solution was centrifuged with

5000 rpm, 900mg of NaCl as an isotonic agent and 160mg of methyl paraoxybenzoate as a preservative were added thereto. The resulting mixture was sterile-filtered in a sterilized room, divided into 20 vials of 5ml, promptly lyophilized at below -40℃, sealed to obtain injectable powder.

Example 3

6g of powdered Pulsatillae Radix, 3g of powdered Ginseng Radix and 0.9g of powdered Glycyrrhizae Radix were added to 100ml of distilled water and extracted for 60 minutes at the temperature of below 60℃ with stirring and with adding distilled water corresponding to the water distilled off. After the extracted solution was centrifuged with 5000 rpm, 900mg of NaCl as an isotonic agent and 160mg of propyl paraoxybenzoate as a preservative were added thereto. The resulting mixture was sterile-filtered in a sterilized room, divided into 20 vials of 5ml, promptly lyophilized at below -40℃, sealed to obtain injectable powder.

Example 4

60g of powdered Pulsatillae Radix, 40g of powdered Ulmaceae cortex and 9g of powdered Glycyrrhizae Radix were added to 1000ml of distilled water and extracted with stirring for 60 minutes at the temperature of below 60℃ with adding distilled water corresponding to the water distilled off. The extracted solution was centrifuged with 5000 rpm and promptly lyophilized at below -40℃ to give 38,150mg of the lyophilized powder.

Example 5

60g of powdered Pulsatillae Radix, 60g of powdered Ulmaceae cortex and 9g of powdered Glycyrrhizae Radix were added to 1000ml of 50%(v/v) ethanol and extracted for 60 minutes at the temperature of 50 - 60℃ with adding the alcohol corresponding to that distilled off. The extracted solution was centrifuged with 5000 rpm and promptly lyophilized at below -40℃ to give 45,150mg of the lyophilized powder.

Example 6

60g of powdered Pulsatillae Radix, 30g of powdered Ginseng Radix and 9g of powdered Glycyrrhizae Radix were added to 1000ml of 50%(v/v) aqueous solution of acetone and extracted for 60 minutes at the temperature of 50 - 60℃ with adding the solvent corresponding to the solvent distilled off. The extracted solution was centrifuged with 5000 rpm and promptly lyophilized at below -40℃ to obtain 34650 mg of the lyophilized powder.

Example 7

6g of powdered Pulsatillae Radix, 4g of powdered Ulmaceae cortex and 0.9g of powdered Glycyrrhizae Radix were added to 100ml of 70%(v/v) ethanol and extracted for 60 minutes at the temperature of below 60℃ with stirring and adding the solvent corresponding to the solvent distilled off. After the extracted solution was centrifuged with 5000 rpm, 900mg of NaCl as an isotonic agent and 160mg of methyl paraoxybenzoate as a preservative were added thereto. The resulting mixture was sterile-filtered in a sterilized room, divided into 20 vials of 5ml, promptly lyophilized at below -40℃ and sealed to obtain injectable powder.

Example 8

10g of powdered Ulmaceae cortex was added to 100ml of 50%(v/v) ethanol and extracted for 60 minutes at the temperature of below 60℃ with stirring. The extracted solution was centrifuged with 5000 rpm, and 900mg of NaCl as an isotonic agent and thereto 160mg of methyl paraoxybenzoate as a preservative were added. The resulting mixture was sterile-filtered in a sterilized room, divided into 20 vials of 5ml, promptly lyophilized at below -40℃, sealed to obtain injectable powder.

Example 9

60g of powdered Pulsatillae Radix, 60g of powdered Ulmaceae cortex and 9g of powdered Glycyrrhizae Radix were added to 1000ml of hexane and extracted for 90 minutes at the temperature of below 60℃ with stirring and adding the hexane corresponding to the amount distilled off. The extracted solution was centrifuged with 5000 rpm and the resulting solution was promptly lyophilized at below -40℃ to obtain the lyophilized powder.

Example 10

Lyophilized extract obtained by the example 4	150mg
Crystalline cellulose	50mg
Lactose	50mg
Magnesium stearate	3mg

The above ingredients were cast into tablets by a conventional method and sealed with aluminium foil

Example 11

Lyophilized extract obtained by the example 6	150mg
Lactose	30mg
Corn starch	30mg
Talc	5mg
Magnesium stearate	3mg

The above ingredients were filled into a hard capsule of gelatin by a conventional method and sealed with aluminium foil

Example 12

Lyophilized extract obtained by the example 5	1000mg
Conventional ointment base	q.s.

The above ingredients were formulated into 10g of ointment, and filled and sealed into an aluminium tube.

Experiment 1

: Acute toxicity

The lyophilized powder obtained by the example 1 was administered to 8 rats of 234-276g, whereby the LD_{50} was 800mg/kg.

Experiment 2

: Antitumor effect

0.1ml of suspension of Sarcoma 180 cells($1x10^6$ cells) was injected (s.c.) into 30 rats of about 25g to develop tumors. After 6 days, 0.15ml

of injection prepared by dissolving the injectable powder of the example 1 in 5ml of distilled water for injection was injected (s.c.) to 10 rats once a day and 0.15ml of injection of the comparative example 1 was injected (s.c.) to another 10 rats once a day. While, 0.15ml of physiological saline solution was injected to the other 10 rats for 10 days as the control group.

Each 9 rats of the groups which were treated with the injection of the example 1 and the comparative example 1 were cured by injection for 15 days, and each 1 rat of the groups died at the 16th day, while 10 rats of the control group died all off beginning at the 10th day till to the 15th day.

Experiment 3

: Antitumor effect

0.1ml of suspension of Sarcoma 180 cells($1x10^6$ cells) was injected(s.c.) into 30 rats of about 25g to develop tumors. After 6 days, 0.15ml of injection prepared by dissolving the injectable powder of the example 1 in 5ml of distilled water for injection was injected (s.c.) to 10 rats (group 1) once a day, and 0.15ml of injection of the comparative example 1 preserved for 6 months in a refrigerator was injected (s.c.) to another 10 rats (group 2) once a day, and 0.15ml of physiological saline solution was injected to the other 10 rats for 10 days as the control group (group 3),

9 rats of the group 1 were cured with injection for 15 days, and 1 rat of the group 1 died at the 17th day. 3 rats of the group 2 were cured by injection for 15 days, 1 rat died at the 12th day, 3 rats died at the 15th day, and 3 rats were died at the 17th day. 10 rats of the group 3 died all off beginning at the 10th day till to the 15th day.

Experiment 4

: Antitumor effect

0.1ml of suspension of Sarcoma 180 cells($1x10^6$ cells) was injected (s.c.) into 48 rats of about 25g to develop tumors. Beginning at the 9th day(terminal stage of cancer), 0.15ml of sample injection prepared by dissolving the injectable powder of the example 3 in 5ml of distilled water for injection was injected (s.c.) to 7 rats once a day (group 1), each 0.15ml of the sample injection of the example 3 was injected (s.c.) to another 7 rats twice a day(group 2), 0.15ml of sample injection of the comparative example 1 was injected (s.c.) to another 7 rats once a day (group 3), each 0.15ml of sample injection of the comparative example 1 was injected (s.c.) to another 7 rats twice a day (group 4), 0.15ml of injection of the comparative example 1 which was preserved for 3 months in a refrigerator was injected (s.c.) to another 7 rats once a day (group 5), each 0.15ml of sample injection of the comparative example 1 which was preserved in a refrigerator for 6 months was injected (s.c.) to another 7 rats twice a day (group 6), and the other 6 rats were used as the control group.

In the group 1 and the group 3, each 1 rat died at the 15th day from carcinogenesis, each 1 rat died at the 17th day, each 1 rat died at the 18th day, and each 1 rat died at the 20th day, while each 3 rats were cured by injection for 20 days (at the 29th day from carcinogenesis).

In the group 2 and the group 4, each 1 rat died at the 16th day from carcinogenesis, each 1 rat died at the 18th day, each 1 rat died at the 19th day, and each 1 rat died at the 21th day, while each 3 rats were cured by injection for 19 days (at the 28th day from carcinogenesis).

In the group 5, 1 rat died at the 15th day of carcinogenesis, 1 rat

died at the 17th day, 1 rat died at the 18th day, 1 rat died at the 19th day, and 1 rat died at the 21th day, while 2 rats were cured by injection for 21 days.

In the group 6, 1 rat died at the 15th day of carcinogenesis, 1 rat died at the 16th day, 1 rat died at the 18th day, 1 rat died at the 19th day, and 2 rats died at the 20th day, while 1 rat was cured by injection for 20 days.

In the group 7, all rats died at the 15th day of carcinogenesis.

Experiment 5

: Clinical Test on a volunteer by administration of the injection prepared by dissolving the injectable powder of the example 1 in distilled water for injection.

Subject :

Name : KIM, Myung-Won (36 years old at the treatment, male)

Address : #6-501, Moran 2cha Apt., Shingi-dong, Dong-gu, Taegu, Korea

Kind of disease : Progressed thyroid cancer

Diagnosis : General hospital affiliated to the Youngnam University in Taegu on May 7, 1992.

Period of medication : From May 16, 1992 to October 20, 1992

Medication : 12ml of the injection was injected (i.v.) once a day for 4 days, while each 10ml of the injection was injected into the protruded tumor twice a day for 8 days (totally 15 times), and then no treatment of injection was performed for 25 days. After that, medication was repeated by i.v. injection and direct injection into the protruded tumor as the same method above, whereby the tumor disappeared

completely. After 5 years, the General hospital affiliated to the Youngnam University and the General hospital affiliated to the Chungnam University decided that the patient was completely cured.

Experiment 6

: Clinical Test on a volunteer by administration of the injection prepared by dissolving the injectable powder of the example 2 in distilled water for injection.

Subject :

Name : KIM, Chul-Ki (50 years old at the treatment, male)

Address : #1223-404, Mokdong Apt., Shinjung-dong, Yangchon-gu, Seoul, Korea

Kind of disease : Progressed lung cancer. Weight loss, Tussis, Bloody phlegm, Dyspnea due to residual cancer after operation.

Diagnosis : Christian Hospital in Wonju affiliated to Yonsei University on May 18, 1989.

Period of medication : From September 3, 1989 to March 5, 1990

Medication : 10ml of the injection was injected (i.v.) once a day for 4 days, and then no treatment was performed for 3 weeks. Same medication was repeated for 7 months of the above period, whereby the tumor and related symptoms disappeared completely. After 6 years, the General hospital affiliated to the Yonsei University and the General hospital affiliated to the Chungnam University decided that the patient was completely cured.

Experiment 7

: Clinical Test on a volunteer by administration of the injection prepared by dissolving the injectable powder of the example 2 in distilled water for injection.

Subject :

Name : SUH, Sang-Bong (35 years old at the treatment, male)

Address : 756, Shibang-ri, Jangmok-myeon, Geohje-gun, Kyungsangnam-do, Korea

Kind of disease : Progressed rectal cancer. Weight loss and severe pain due to residual cancer after partial removal of tumor by operation.

Diagnosis : Goshin Medical Center in Pusan on July 19, 1991.

Period of medication : From November 19, 1991 to May 12, 1992

Medication : 12ml of the injection was injected (i.v.) once a day for 4 days, and then no treatment was performed for 3 weeks. Same medication was repeated for 7 months of the above period, whereby the residual tumor disappeared completely. After 6 years from the medication, the Goshin Medical Center in Pusam and the General hospital affiliated to the Chungnam University decided that the patient was completely cured.

Experiment 8

: Clinical Test on a volunteer by administration of the injection prepared by dissolving the powder of the example 3 in distilled water for injection.

Subject :

Name : PARK, Ju-Sang (45 years old at the treatment, male)

Address : 15/2, 387-3, Bugok 1-dong, Kumjeong-gu, Pusan, Korea

Kind of disease : Progressed stomach cancer. Feeling heavy on the stomach, Dyspepsia and Weight loss.

Diagnosis : Goshin Medical Center in Pusan on December 29, 1989

Period of medication : From March 1, 1990 to September 29, 1990

Medication : 12ml of the injection was injected (i.v.) once a day for 4 days, and simultaneously each 173mg of the sample was taken internally four times a day. The i.v. injection was perform in such a manner that the injection was i.v. injected for 4 days and then no injection was made for 4 weeks, while the internal administration was continued. As the result, the symptoms disappeared completely and he was ascertained to be normal by the biopsy. After 6 years, the Goshin Medical Center decided that the patient was completely cured.

Experiment 9

: Clinical Test on a volunteer by administration of the injection prepared by dissolving the powder of the example 5 in distilled water for injection.

Subject :

Name : LEE, Bok-Do (65 years old at the treatment, male)

Address : 322-81, Chosan-ri, Hwagok-myeon, Yangsan-gun, Kyungsangbuk-do, Korea

Kind of disease : Progressed liver cancer. Abdominal dropsy, dyspepsia and weight loss due to residual cancer after partial removal of tumor by operation.

Diagnosis : Goshin Medical Center in Pusan on April 8, 1994.

Period of medication : From May 31, 1994 to December 30, 1994

Medication : 9ml of the injection was injected (i.v.) once a day for 4 days, and simultaneously 4ml of the sample was injected directly into the residual tumor in liver once a day for 3 days and then for 3 weeks no treatment was performed. The same medication was performed three times, and then from the 4th cycle of medication, only i.v. injection was performed, whereby the residual tumor disappeared completely. After 3 years, the Goshin Medical Center and the Hospital affiliated to Chungnam University decided that the patient was completely cured.

Stability test 1

Samples of the lyophilized power for injection prepared by the examples according to the present invention were preserved for 2 years and then dissolved in distilled water for injection. The resulting solution was transparent light brown and no precipitation settled.

Stability test 2

Samples of injection prepared by the comparative example 2 were stored for 1 month, 2 months and 3 months respectively. In the resulting solutions precipitate settled in 1 month and in three months the solutions was turbid whereby it was unable to be used for injection.

Effects of the invention

As seen from the above experiments, the antitumor compositions by the prior invention are sensitive to moisture, and even if stored in a refrigerator, they are easily deteriorated and efficacy thereof is severely decreased, whereby they cannot be preserved for a long time and then

they cannot be used as a medicine.

However, the present compositions which are prepared by extracting the herb ingredients at the temperature of below 60℃ and after extraction immediately lyophilizing the extract have no change in quality and efficacy thereof after long preservation, whereby they may be used safely.

Claims

1. A pharmaceutical composition having antitumor activity prepared by extracting 0-100wt% of powdered Pulsatillae Radix and 0-100wt% of powdered Ulmaceae cortex in a solvent at the temperature of below 60℃, filtering and lyophilizing the extract, and then admixing the lyophilized powder with conventional auxiliaries, or admixing the above extracted solution with auxiliaries, then filtering and lyophilizing the mixture, and then formulating the lyophilized powder to a pharmaceutical preparation by a conventional method used in the pharmaceutics.

2. A pharmaceutical composition having antitumor activity prepared by extracting 0-100wt% of powdered Pulsatillae Radix and 0-100wt% of powdered Ulmaceae Cortex, provided that the content of Pulsatillae Radix and Ulmaceae Cortex is over 30wt%, and one ore more ingredients selected from 0-70% of powdered Ginseng Radix and 0-70wt% of Glycyrrhizae Radix in a solvent at the temperature of below 60℃, filtering and lyophilizing the extract, and admixing the lyophilized powder with conventional auxiliaries, or admixing the extracted solution with auxiliaries, filtering and lyophilizing the mixture, and then formulating the lyophilized powder to a pharmaceutical preparation by a conventional method used in the pharmaceutics.

3. The pharmaceutical composition according to claims 1 or 2, wherein the solvent is selected from water, alcohol, acetone, ethyl acetate and mixtures thereof and the composition is formulated in a form selected from powder, granule, tablet, capsule, injectable powder and ointment.

4. The pharmaceutical composition according to claims 1 or 2, wherein the auxiliaries are one or more selected from diluent, binding agent, disintegrator, preservative, indolent, isotonic agent and lubricant.

5. A process for the preparation of a pharmaceutical composition having antitumor activity comprising extracting 0-100wt% of powdered Pulsatillae Radix and 0-100wt% of powdered Ulmaceae cortex in a solvent at the temperature of below 60℃, filtering and lyophilizing the extract, and admixing the lyophilized powder with conventional auxiliaries, or admixing the above extracted solution with conventional auxiliaries, then filtering and lyophilizing the mixture, and then formulating the lyophilized powder to a pharmaceutical preparation by a conventional method used in the pharmaceutics.

6. A process for the preparation of a pharmaceutical composition having antitumor activity comprising extracting 0-100wt% of powdered Pulsatillae Radix and 0-100wt% of powdered Ulmaceae cortex, provided that the content of Pulsatillae Radix and Ulmaceae Cortex is over 30wt%, and one ore more ingredients selected from 0-70wt% of powdered Ginseng Radix and 0-70wt% of powdered Glycyrrhizae Radix in a solvent at the temperature of below 60℃, filtering and lyophilizing the extract, and admixing the lyophilized powder with conventional auxiliaries, or admixing the above extracted solution with auxiliaries, then filtering and lyophilizing the mixture, and then formulating the lyophilized powder to a pharmaceutical preparation by a conventional method used in the pharmaceutics.

7. The process according to claims 5 or 6, wherein the solvent is

selected from water, alcohol, acetone, ethyl acetate and mixtures thereof and the composition is formulated in a form selected from powder, granule, tablet, capsule, injectable powder and ointment.

8. The process according to claims 5 or 6, wherein the auxiliaries are one or more selected from diluent, binding agent, disintegrator, preservative, indolent, isotonic agent and lubricant.

Abstract

The present invention relates to a pharmaceutical composition having antitumor activity and comprising Pulsatillae Radix and/or Ulmaceae cortex, and more particularly the composition may be prepared by extracting powdered Pulsatilla Radix and/or powdered Ulmaceae cortex, and optionally one ore more ingredients selected from powdered Ginseng Radix and Glycyrrhizae Radix in a solvent at the temperature of below 60℃, filtering and lyophilizing the extract, or admixing the above extracted solution with conventional auxiliaries, then filtering and lyophilizing the resulting mixture, and then formulating the lyophilized powder thus obtained to a pharmaceutical preparation by a conventional method used in the pharmaceutics.

The present antitumor composition is stable and maintains efficacy even if it is preserved for several years.

FOR UTILITY/DESIGN
CIP/PCT NATIONAL/PLANT
ORIGINAL/SUBSTITUTE/SUPPLEMENTAL
DECLARATIONS

RULE 63 (37 C.F.R. 1.63)
DECLARATION AND POWER OF ATTORNEY
FOR PATENT APPLICATION
IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

PM & S FORM

As a below named inventor, I hereby declare that my residence, post office address and citizenship are as stated below next to my name, and I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the INVENTION ENTITLED PHARMACEUTICAL COMPOSITION HAVING ANTITUMOR ACTIVITY AND PROCESS FOR THE PREPARATION THEREOF

the specification of which (CHECK applicable BOX(ES))

X BOX(ES) →
A. ☐ is attached hereto.
B. ☐ was filed on ____ as U.S. Application No. ____ /
C. ☒ was filed as PCT International Application No. PCT/ KR99/00659 on 3 November, 1999

and (if applicable to U.S. or PCT application) was amended on

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above. I acknowledge the duty to disclose all information known to me to be material to patentability as defined in 37 C.F.R. 1.56. Except as noted below, I hereby claim foreign priority benefits under 35 U.S.C. 119(a)-(d) or 365(b) of any foreign application(s) for patent or inventor's certificate, or 365(a) of any PCT International Application which designated at least one other country than the United States, listed below and have also identified below any foreign application for patent or inventor's certificate, or PCT International Application, filed by me or my assignee disclosing the subject matter claimed in this application and having a filing date (1) before that of the application on which priority is claimed, or (2) if no priority claimed, before the filing date of this application

PRIOR FOREIGN APPLICATION(S)

Number	Country	Day/MONTH/Year Filed	Date first Laid-open or Published	Date Patented or Granted	Priority NOT Claimed
1998/47025	KR	03/11/1998			
1998/48277	KR	11/11/1998			

If more prior foreign applications, X box at bottom and continue on attached page.

Except as noted below, I hereby claim domestic priority benefit under 35 U.S.C. 119(e) or 120 and/or 365(c) of the indicated United States applications listed below and PCT international applications listed above or below and, if this is a continuation-in-part (CIP) application, insofar as the subject matter disclosed and claimed in this application is in addition to that disclosed in such prior applications, I acknowledge the duty to disclose all information known to me to be material to patentability as defined in 37 C.F.R. 1.56 which became available between the filing date of each such prior application and the national or PCT international filing date of this application

PRIOR U.S. PROVISIONAL, NONPROVISIONAL AND/OR PCT APPLICATION(S)

Application No. (series code/serial no.)	Day/MONTH/Year Filed	Status pending, abandoned, patented	Priority NOT Claimed

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

And I hereby appoint Pillsbury Madison & Sutro LLP, Intellectual Property Group, 1100 New York Avenue, N.W., Ninth Floor, East Tower, Washington, D.C. 20005-3918, telephone number (202) 861-3000 (to whom all communications are to be directed), and the below-named persons (of the same address) individually and collectively my attorneys to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith and with the resulting patent, and I hereby authorize them to delete names/numbers below of persons no longer with their firm and to act and rely on instructions from and communicate directly with the person/assignee/attorney/firm/ organization who/which first sends/sent this case to them and by whom/which I hereby declare that I have consented after full disclosure to be represented unless/until I instruct the above Firm and/or a below attorney in writing to the contrary.

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(Include Zip Code)			

FOR ADDITIONAL INVENTORS, "X" box ☐ and proceed on the attached page to list each additional inventor.

☐ See additional foreign priorities on attached page (incorporated herein by reference).

Atty. Dkt. No. PM ____
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Rule 56(a) & (b) = 37 C.F.R. 1.56(a) & (b)
PATENT AND TRADEMARK CASES - RULES OF PRACTICE
DUTY OF DISCLOSURE

(a ...Each individual associated with the filing and prosecution of a patent application has a duty of candor and good faith in dealing with the [Patent and Trademark] Office, which includes a duty to disclose to the Office all information known to that individual to be material to patentability...(b) information is material to patentability when it is not cumulative and (1) It also establishes by itself, or in combination with other information, a prima facie case of unpatentability of a claim or (2) refutes, or is inconsistent with, a position the applicant takes in: (i) Opposing an argument of unpatentability relied on by the Office, or (ii) Asserting an argument of patentability

PATENT LAWS 35 U.S.C.

§102. Conditions for patentability; novelty and loss of right to patent

A person shall be entitled to a patent unless--

(a) the invention was known or used by others in this country, or patented or described in a printed publication in this or a foreign country, before the invention thereof by the applicant for patent or

(b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of the application for patent in the United States, or

(c) he has abandoned the invention, or

(d) the invention was first patented or caused to be patented, or was the subject of an inventor's certificate, by the applicant or his legal representatives or assigns in a foreign country prior to the date of the application for patent in this country on an application for patent or inventor's certificate filed more than twelve months* before the filing of the application in the United States, or

(e) the invention was described in a patent granted on an application for patent by another filed in the United States before the invention thereof by the applicant for patent, or on an international application by another who has fulfilled the requirements of paragraphs (1), (2), and (4) of section 371(c) of this title before the invention thereof by the applicant for patent, or

(f) he did not himself invent the subject matter sought to be patented, or

(g) before the applicant's invention thereof the invention was made in this country by another who had not abandoned, suppressed, or concealed it. In determining priority of invention there shall be considered not only the respective dates of conception and reduction to practice of the invention, but also the reasonable diligence of one who was first to conceive and last to reduce to practice, from a time prior to conception by the other.

§103. Condition for patentability; non-obvious subject matter

(a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

(c) Subject matter developed by another person, which qualified as prior art only under subsection (f) or (g) of section 102 of this title, shall not preclude patentability under this section where the subject matter and the claimed invention were, at the time the invention was made, owned by the same person or subject to an obligation of assignment to the same person.

* Six months for Design Applications (35 U.S.C. 172).